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Expression of Cellulose synthase fused to Green Fluorescent Protein in the Tobacco Suspension-cultured Cells

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Introduction

Cellulose microfibril is the major constituent of the plant cell wall. It is clear that cellulose biosynthesis occur in a terminal complex (TC) located in a plasma membrane¹⁾. However, nobody has proved that TCs move in the plasma membrane. Our purpose is to prove whether rosette TCs move in the plasma membrane of higher plant cells or not. We tried to observe a movement of cellulose synthase labeled with green fluorescent protein (GFP) on the plasma membrane²⁻⁴⁾.

Materials and Methods

Tobacco BY-2 suspension cultured cells were maintained in Linsmaier and Skoog (LS) medium supplemented with 3% sucrose and 1 μ M 2, 4-D in 100 ml Erlenmayer flask on a shaker at 27°C in the dark. Three-day-old suspension cultured cells were used. We used a partial *GhCesA3* gene (GeneBank accession number AF150630) that contains 1.4 kb starting from 5' end that code cellulose synthase derived from cotton during secondary wall synthesis. An eukaryote codon-optimized synthetic S65T GFP (Niwa *et al.* 1999) was the generous gift of Dr. Y Niwa (University of Shizuoka, Shizuoka, Japan).

The *GhCesA3* gene fused to GFP (*GhCesA GFP* vector) was constructed in pUC18 vector contained Califlower mosaic virus 35S promoter (CaMV35S), syntheticGFP (S65T) and nopaline synthase terminator. The *GhCesA3* gene was inserted in downstream of CaMV35S promoter. The *GhCesA3: GFP* vector and control GFP vector were introduced into tobacco BY-2 cells by particle delivery system (PDS-1000/He Particle Delivery System, Bio-Rad). For microscopic observation, we used confocal lazer scanning microscope (LSM5Pascal, Carl Zeiss).

Results and Discussion

First, we tried to introduce control GFP vector into tobacco BY-2 cells. After 3 hours, GFP fluorescence was recognized. After 6 hours, the fluorescence became more

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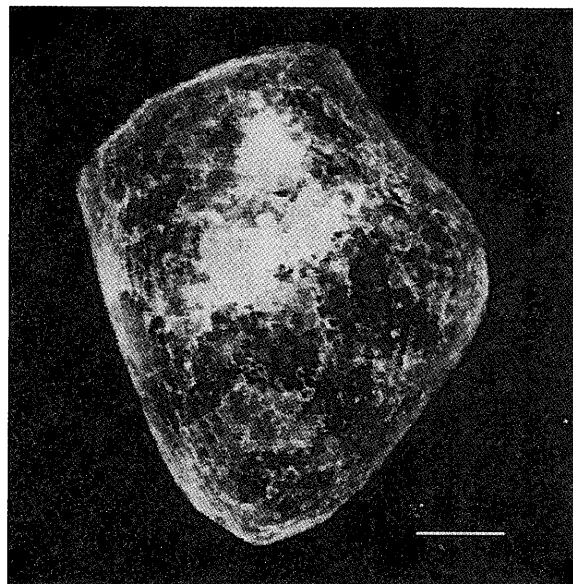


Fig. 1. Localization of CesA: GFP fusion protein in Tobacco BY-2 cells. Bar=10 μ m.

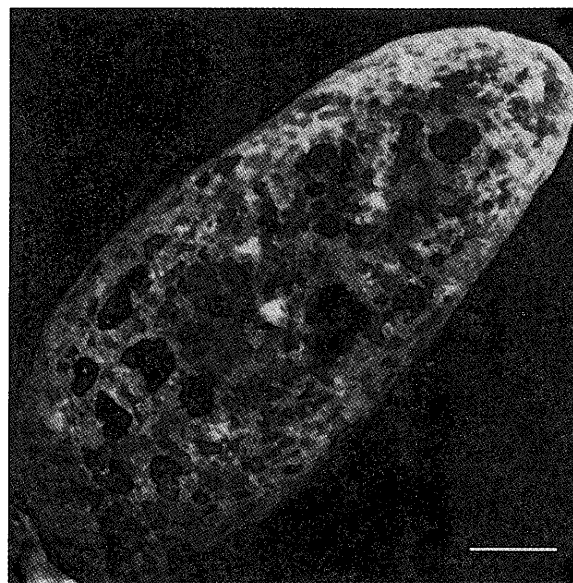


Fig. 2. DiOC6-stained 3D view of Tobacco BY-2 cells. Bar=10 μ m.

clear. GFP signal was located in whole cytoplasm and nucleus. No signal was located in a particular organelle (data not shown).

Subsequently, we tried to introduce *CesA3: GFP* vector into tobacco BY-2 cells. After 3 hours, *CesA3: GFP* fusion protein was expressed in the cells. Although GFP fluorescence was very weak, it was visualized as an outline of nucleus and near plasma membrane. After 6 hours, a network with a green color was recognized as outline of nucleus and near plasma membrane. The pitch of network was in the range from 1 to 2 μm (Fig. 1). After 9 hours, this network became more clear. From this stage, some cells showed both a rough network and a small particle fluorescence. The pitch of rough network was in the range from 2 to 5 μm , the small particle fluorescence was in the range from 1 to 5 μm .

Because cellulose synthase is a transmembrane protein, it was expected that *CesA3: GFP* fusion protein was expressed at endoplasmic reticulum (ER) and transferred from ER to plasma membrane via Golgi apparatus. In

order to confirm that the network is made of ER, we compared *CesA3: GFP* fluorescence pattern with DiOC₆ dyeing pattern. DiOC₆ is a dye that specifically stain the ER (Fig. 2). *CesA3: GFP* fluorescence pattern was consistent with DiOC₆ dyeing pattern. As a result, it was considered that the network was ER and *CesA3: GFP* fusion protein was expressed at the ER. The small particle fluorescence was closely located on the ER, and the signal's diameter was in the range from 1 to 5 μm . They were recognized after 9 hours for the first time. These findings suggest that *CesA3: GFP* fusion protein could be located at Golgi apparatus.

Reference

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